

CROONIAN LECTURE.—*On the Structural Constituents of the Nucleus, and their Relation to the Organisation of the Individual.*

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The latter half of the nineteenth century witnessed three events of supreme importance to biology. The first of these consisted in that reasoned theory of the mode of origin of new species with which the name of Charles Darwin will always remain associated. The second lay in the discovery, made by Strasburger in 1875, that the nucleus is not only a permanent organ of the cell, but that certain definite constituents of it are transmitted in unbroken sequence from one cell generation to another. Thirdly, Oscar Hertwig, also in 1875, showed that fertilisation consists not only in the union of male and female cells, but that the union of the two nuclei forms an essential part of the process.

At the present time, when evolutionary problems are being attacked at their very roots by the experimental study of variation, results are being accumulated which are capable of being dealt with from a cytological standpoint. Much is to be expected from a joining together of the forces engaged on what are really only different aspects of the same problem. What we really want to know is the nature and mode of working of the machinery which is responsible for the appearance of the characters manifested, as well as inherited, by the organism. We also are concerned with the nature of those inner changes which find their outward expression in what we designate as variation.

Whilst I do not of course pretend that all these questions can be satisfactorily answered at present, so many new facts which bear upon them have come to light within recent times that I venture to indicate some of what I conceive to be the more important results that have been obtained.

Every animal and plant either consists of a single cell, or it is made up of a cell-colony the members of which have had a common origin, inasmuch as they have all sprung from one mother-cell, the egg. And it is in the cells that the properties of an individual must ultimately be sought.

The more closely the cell is studied, the more irresistibly are we compelled to admit the supreme importance of the nucleus in directing and controlling its metabolic activities. Experiments have abundantly proved that if a nucleated protoplasmic mass be divided by artificial means, so that one

portion contains, while the other lacks, the nucleus, the former alone is able to continue to discharge the ordinary vital functions, and to manifest those chemical and physical properties which necessarily underlie them. The enucleated fragment, on the other hand, although it may persist for some time, nevertheless loses, more or less rapidly, all those special faculties which should enable it to maintain a separate existence. Synthetic and secretory activities disappear, and death sooner or later supervenes.

Seeing, then, that the nucleus plays so fundamental a part in maintaining the due sequence of chemical change in the cell, the question naturally arises as to whether all those properties of the individual which find their expression in form, and in other externally recognisable characters, may not be attributed to the interaction of the nucleus with the cytoplasm external to it; whether they are not merely the inevitable result and outcome of the series of material changes that succeed each other within the confines of the constituent cells. I believe the answer must be in the affirmative. At the present time, however, such a general statement has but little value beyond expressing the general direction in which the many lines of investigations appear to be advancing. We are not likely, within the near future, to be in a position to trace all the stages which intervene between the importing of raw material and the turning out of the finished fabric. Modern research clearly indicates that the proximate problems are more intricate than they were formerly supposed to be. Nevertheless, I venture to think that the very complexity of the regulatory processes emphasises, rather than diminishes, the probability that a comprehensive physical explanation of organisation and inheritance will one day fall within our reach. But the final synthesis can only be made possible when we have understood the details, when the more immediate problems have been clearly stated and successfully solved.

Some of these problems are already in process of solution; thus numerous investigators during the last 30 years have done much to clear up the essential features of fertilisation, although it cannot be said that as yet there exists complete agreement on all points. For while it has been generally conceded since Hertwig's discovery, already alluded to, that the nuclear, and not the cytoplasmic, association constitutes the significant part of the process, there are still some who hold that the fusion of the cytoplasm is of direct and material importance in the transmission of the hereditary properties of the individual.

As this is a matter of great weight, an examination of the facts yielded by a study of fertilisation is demanded. It is true that the male gamete carries with it into the egg a small amount of cytoplasm. On penetrating the egg,

the nucleus escapes from its cytoplasmic investment which then disintegrates and mixes with that of the egg, whilst the two nuclei approximate and often fuse. Although the amount of cytoplasm thus introduced is exceedingly minute, as compared with that of the egg, probably no one at the present time would regard this circumstance as *necessarily* implying a correspondingly small degree of importance, unless other evidence pointed also to the same conclusion.

Boveri has shown that when an enucleated fragment of the egg of one species of echinoderm is fertilised by the sperm of another species, or even genus, the resulting larva resembles the male parent. These statements have been sometimes challenged, but the explanation of the discrepant results obtained by different observers probably lies in the fact that the eggs themselves may be readily stimulated to develop parthenogenetically. Enucleated fragments of eggs already in this condition, even in the earliest stages, will have already received a developmental stimulus of a material nature from their own (*i.e.*, the female) nucleus. This must inevitably produce an effect on the final result. The recent discovery\* that if eggs which are *known* to be in the early stages of parthenogenetic development are fertilised by the sperm of another species, the offspring actually do show a markedly increased resemblance to the maternal parent, is of the highest theoretical interest in connection with the mode of interaction between the nucleus and the cytoplasm.

The entrance of more than one sperm, and the fusion of their nuclei with that of the egg, is commonly followed by great disturbance in the course of development of the embryo, if, indeed, it is able to proceed at all. It is remarkable how very seldom polyspermy occurs in healthy cultures. I have myself examined many thousands of the eggs of *Fucus*, and I have only seen two or three examples of the entrance of more than one sperm into each egg. In some cases, however, polyspermy can be readily induced by anaesthetising, or in other ways interfering with the normal conditions of the egg; and the abnormal results that ensue serve to emphasise the correlation existing between nuclear constitution, and cell- and tissue-development.

The rarity of polyspermy, at any rate amongst the majority of plants, points to the existence of some means of actively preventing its occurrence in ordinary eggs. It is often stated that a cell wall is instantly excreted over the surface of the egg on fertilisation, and that in this way the entrance of additional sperms is prohibited. Such an explanation, if, indeed, it ever suffices, will not cover every case. In some eggs, as, for example, those of the *Fucaceæ*, the membrane is not formed till an appreciable time has elapsed

\* Curt Herbst, "Vererbungsstudien, IV," 'Arch. f. Entw.-Mech. d. Organismen,' vol. 22.

after fertilisation. Hence the reason for the exclusion of the extra sperms must be sought in another direction. It seems probable that the cause of exclusion is connected with a sudden chemical change immediately resulting from the entrance of the first sperm into the cytoplasm of the egg. I shall show that in one case, at any rate, there is direct evidence of such a change in the reaction of the egg, and it may be remarked that it is quite intelligible that such a reaction should be affected or even arrested by anæsthetics, or other substances that interfere with the proper metabolism of the cell.

In the first place, it is certain that sperms are attracted to the egg by specific substances which are excreted from it and diffuse into the surrounding medium. Different sperms are affected by different substances, and what will attract those of one plant may be entirely without influence on those of another. For example, if two vaccine tubes, filled with very weak solutions of sugar and malic acid respectively, be placed in a vessel containing a mixture of the actively motile sperms of a moss and of a fern, those of the former will crowd into the tube containing the cane sugar, those of the fern into that filled with the solution of malic acid.

Since, then, the swarming of the sperms towards the egg is effected as a response to a definite chemiotactic stimulus, it is inherently probable that a correspondingly negative or repellant stimulus provides the egg with the means securing it against the entrance of more than one sperm. We have been able to show that this is the case in several of the Fucaceæ. If the large eggs of *Halidrys* be allowed to extrude into sea-water together with the sperms, the latter will, very shortly, be found to congregate on the surface of the egg. Each sperm possesses two cilia, with one of which it holds on to the egg, whilst with the other it lashes the water, endeavouring to force an entrance through the limiting layer of the protoplasm. So active are the movements that the relatively huge egg is seen to be rocked about in the water. In a short time a sudden change takes place in the egg. It swells, and then in a moment it becomes rugose or prickly first at one spot, and thence rapidly over its whole surface. At the same instant, all the sperms that were moving over its periphery rapidly leave it, and those that are unable to detach themselves sufficiently quickly are seen to become immediately paralysed and to die. Soon afterwards the egg shrinks in size and becomes quite smooth again. Later on a membrane is excreted over its surface, proving definitely that fertilisation has occurred.

In this instance, then, the case for the emission of a repellant, and even lethal, substance from the surface of the egg as the result of fertilisation is quite clear, and it perhaps diffuses from the fine mucilage-like threads that are shot out from the rugose periphery. Since making these observations on

Halidrys, I have been able, though not so easily, to detect the repellant effect in some other Fucaceæ. Still more recently, in an investigation on ferns involving the details of fertilisation, we have observed that after one sperm had entered the egg, the others close to its surface showed signs of greater disorganisation than those which were further removed from it. In this instance also, then, it seems clear that the entrance of a sperm produces a chemical change in the egg which is reflected over its whole surface, although we are not as yet in a position to make any definite statement as to the precise nature of the substance concerned.

It is another question whether the change is produced directly in the cytoplasm, or whether it emanates in the first place from the nucleus. Although certain facts seem at first sight to favour the latter view, I think the former is the more correct one. In many gymnosperms the two sperms which are formed in the pollen tube are both extruded into the egg. But only one of them is functional; the other soon disintegrates in the cytoplasm, and does not approach the egg-nucleus. But it must be borne in mind that the sperms are rather passively *shot into* the egg from the pollen-tube, than individually *attracted* into it. In Cycads, however, where the sperms are actively motile, only one enters the egg as a rule, and when more do succeed in penetrating it, they always perish at once. It may be suggested that as the egg of these plants is of enormous size as compared with that of the Fucaceæ, the change which is produced instantaneously in the latter may take longer to complete in the case of the gymnosperms. In any event it *does* take place, and the destruction of the additional sperms occurs in time to prevent them getting near the nucleus of the egg. Further evidence in favour of the cytoplasmic origin of the repellant substance is again afforded by Halidrys. It often happens that the eggs, as they are being shed, are broken into two pieces, of which one is destitute of a nucleus. Such fragments, however, as Williams has shown, commonly attract the sperms, and on the entrance of one of them the same repellant influence on the rest is as evident as in the case of normal eggs. Wilson, also, found conclusive evidence to show that when enucleated fragments of echinoderm-eggs are fertilised, only a single sperm is concerned in the process.

These observations seem to me to prove that the essential act of fertilisation consists not only in the union of two, and not more than two, nuclei, but also in the retention by them of a definite *structural basis*. For we have seen that the mere addition of nuclear *material* cannot be regarded as the important act, inasmuch as any extra sperms entering the egg are without influence on development, prejudicial or otherwise, provided that, as in the

gymnosperms, they are destroyed so that their structural individuality is lost. That the egg-cytoplasm is essential as building-material is, of course, not disputed, and in so far as it consists of different substances, these will have different prospective values in the final result. In the case of many animal eggs, the cytoplasm shows physical differences in its various regions, and there is reason to think that this diversity in material is, at any rate sometimes, connected with differences in the structures finally to be developed. If, then, the egg be mutilated, so that a definite kind of raw material is abstracted from it, an imperfect embryo might be looked for, unless the removed raw material could be regenerated. The nucleus, as architect, would otherwise only be able to build a body correspondingly defective in certain parts. But such considerations as these do not weaken the position of the nucleus as the agent responsible for the particular direction and control of development which is followed by a cell, or, collectively, by a group of cells. In short, just as we have the strongest grounds for associating secretory and other metabolic activities of the cell with the nucleus as the prime mover, so, also, various lines of evidence indicate the same body, or rather certain constituents of it, as guiding the course of those chemical transformations in the cell and in the organism which make themselves apparent in characters such as form, colour, and the like, which can be appreciated by our senses.

I have indicated the possibility that certain constituents of the nucleus, rather than this body as a whole, may be charged with the control and direction of cellular development. It remains therefore to consider the evidence on which such a view, which in some form or another has often been put forward, is based. Darwin, Weismann, De Vries, and others, have each suggested the existence of particles which are responsible for the characters of the individual. And I may at once say that, apart from some such material units, I am unable to understand how the large mass of facts of which we are now in possession can be explained.

Within recent times our knowledge respecting the results of breeding, and also respecting the details of cellular organisation, has been greatly widened, and I believe that the evidence thus drawn from two entirely different sources irresistibly points to the same conclusion, and that the existence of discrete units which the breeder, on purely theoretical grounds, has been compelled to assume, is entirely borne out by the observed presence of certain structures which exist in vast numbers in the nucleus. These structures enter into the composition of those remarkable bodies, the chromosomes, which form such striking objects in the nucleus at the periods of division. Of course, I need not say that the particles in question are not to

be regarded as the immediate characters themselves; they may, perhaps, be roughly compared with that class of bodies known as ferments, setting up in the extra-nuclear cytoplasm those changes which, in their sum-total, constitute development, and producing those chemical re-arrangements of which form, colour, etc., are the visible expression.

It will be convenient, in the first place, to examine the results obtained from experiments in cross-breeding, a field in which Bateson and others have achieved so great a measure of success. We can afterwards try to relate these results with the salient facts of nuclear structure which have been elucidated within recent times.

If two pure parents which differ in, say, one character from each other be chosen and mated, the offspring almost always behaves in a definite and uniform manner with regard to the character in question. Often it appears as if they all inherited the character of one parent only, that of the other having been entirely lost. But on allowing the hybrids to interbreed, it soon becomes plain that the alternative character was only latent, and not lost at all. It reappears in a fixed percentage of the offspring, usually to the extent of 25 per cent. When these individuals, thus exhibiting the recessive character, are mated, they continue to breed true, and the alternative dominant character, which replaces the recessive one in 75 per cent. of the individuals of that generation, never reappears amongst the recessive progeny so long as they interbreed only with one another.

As regards the individuals which make up 75 per cent. of the second (the  $f_2$ ) generation, it is found that whilst all of them show the dominant character exclusively, only one-third continues to breed true, whilst two-thirds of them, when mated together, prove to be hybrids; and in the next generation these continue to throw out one-quarter pure dominants, one-quarter pure recessives, and one-half hybrids. This is a remarkable result, and although it is not always directly or obviously obtained, it occurs in so large a number of instances as to prove sufficiently that the characters in question do behave as independent units. It is not surprising that there should be apparent exceptions to the rule; some of them have already been shown to be due to an incomplete analysis of the real characters themselves, so that composite features have been mistaken for units. But, at any rate, in the presence of so large a number of cases which *do* conform to the Mendelian rule, they may be safely left on one side so far as our present purpose is concerned, which is to find an explanation of the numerous positive instances that are everywhere recognised.

It is possible to make certain statements, which almost partake of the nature of inevitable conclusions, from a consideration of the mode of

behaviour of those alternative characters so well termed *allelomorphs* by Bateson. In the first place, it is difficult to escape from the inference that the unit characters must be due to *material primordia* which are ultimately responsible for their appearance, and that these several primordia have a separate and persistent individuality of their own. The union of the primordia at fertilisation, as judged by the results, rather resembles a mechanical mixture than the formation of a chemical compound.

Inasmuch as two sexual nuclei unite at fertilisation, each one carrying with it characters of that parent from which it originated, it is clear that each sexual cell or gamete must possess, in its nucleus, half the whole lot of the primordia that will be contained in a fertilised egg. Furthermore, the manner in which the characters are distributed amongst the members of any one generation is such that no sexual cell can contain more than a single individual of any given pair of *allelomorphs* which are present in the body of the organism producing the sexual cells. This circumstance is expressed by the term "Purity of gametes," and it implies that, as regards any particular character, the primordium contributed by one parent only will be present in a gamete, the other primordium or *allelomorph* having been distributed to another gamete. When two gametes unite having identical primordia of any character, the resulting offspring must be pure bred as regards that character, and their progeny, if interbred, will keep true. But this is only so for each *separate* character; the *different* characters behave quite independently of one another. Thus, if we take *Aa* as representing one pair of *allelomorphs*, and *Bb* as representing another distinct pair, no gamete can contain *A* together with *a*, or *B* together with *b*, but it can contain *either* member of each pair, *i.e.*, *AB*, *Ab*, *aB*, or *ab*. When two of these gametes unite, it can easily be seen that the characters will be combined as I have already stated.

The assumption as to the purity of gametes, which is in accordance with the facts it attempts to explain, is in harmony with cytological observations, and therefore may be accepted as representing more than a mere working hypothesis.

The clear results of statistical investigations of this kind seem to me to completely dispose of a view of fertilisation, otherwise quite possible, as being of the nature of a chemical union of the fusing nuclei. The facts conclusively point to the inference that there is only a mechanical mixture of the structural units contained in each of the sexual nuclei that take part in the act of fertilisation; the units retain their own identity, and they are again sorted out, though in different combinations, in connection with the differentiation of the sexual cells for the next generation.

We may now turn to consider the structure of the nucleus, and enquire



into the nature of the evidence for the existence of permanent structures within it which will agree with what seems to be demanded by the facts of heredity as elucidated by experiments on breeding.

The resting nucleus is externally limited by a membrane, within which is contained a gelatinous, more or less reticulated, framework, the linin; and this is bathed in a more fluid medium, the paralinin. There are also one or more spherical bodies, the nucleoli, which form storehouses of materials that become separated and used up during times of nuclear activity. As long as the nucleus is in the resting condition, it betrays little of that intricate structure which it exhibits at periods of its division. Much of the complexity apparent at those times must be attributed to temporary physical changes proceeding in the colloidal masses which are then undergoing rapid change of state; but underlying these there is a permanent structural configuration which is constantly preserved and is a determining factor in controlling the course of those cellular changes on which the organisation of the individual ultimately depends.

When a nucleus is about to enter on division, the linin becomes impregnated with a substance termed chromatin, belonging to the nuclein class of compounds. The chromatin, or its precursors, is mainly to be found in the nucleoli as long as the nucleus is at rest; it is only during the period of division that it becomes aggregated in the linin. But the chromatin is not *evenly* distributed through the linin. It appears as very regularly arranged discontinuous swellings in the thread. These small swellings, formed of chromatin and linin, have been called *chromomeres*. The linin, made up of alternate discs of chromatin- and non-chromatin-containing segments, now either forms a convoluted filament, which finally breaks up into a definite number of segments, the *chromosomes*; or else these bodies are visible at the first onset of division as discontinuous areas of linin mapped out by the accumulation of chromatin in groups of chromomeres. The chromosomes are commonly stated to be constant in number for a given organism, but this is certainly not universally true, especially in the vegetative, or somatic cells. On the other hand, in the reproductive cells the number is on the whole very uniform, and even peculiarities of form distinguishing certain chromosomes may continually recur.

The chromosomes then take up definite positions at the equator of the nucleus, and each chromomere in the thread has already split into two exactly similar halves. This carries with it the longitudinal fission of each chromosome as a whole, and the two halves then move apart and are finally distributed, one to each of the two daughter nuclei which are thus formed.

The chromosomes are the only parts of the original nucleus of which it

can be certainly affirmed that they are equally shared between the two daughter-nuclei. Seeing, then, that the net result of the process is to accurately divide the chromosomes (and consequently the chromomeres) by longitudinal fission, it would seem to be immaterial whether the actual numbers of these bodies remained constant or not, at any rate in the somatic divisions. The uniformity so commonly observed, especially when coupled with the occasional departure from it, probably indicates that the number typically present is to be taken as an expression of the organising function of the cell as a whole, rather than as evidence of the independent nature of the chromosomes themselves.\* And this point of view is intelligible when we reflect that they are themselves only organised bundles of chromomeres.

At fertilisation, two nuclei unite, and their chromosomes consequently mingle. The immediate descendants of the cell thus produced are all similarly provided with double the number of chromosomes contained in the nuclei of the sexual cells before fusion occurred. It is, of course, clear that, in any organism, a limit to such duplication must soon be reached. It is found, as a matter of fact, that each act of fertilisation, with its concomitant doubling of the chromosomes, is associated with a correlative process of reduction to one-half. To this process the name of *Meiosis*† has been given. The way in which meiosis is effected is surprisingly uniform in both animals and plants, and its physiological importance may be gathered from the fact that, whilst it occurs in every organism that reproduces itself sexually, it is not bound up with morphological periods in the life history. It antedates all morphological differentiation, and is only *directly* related to sexuality, of the continuance of which it is both a consequence and a condition.

The great theoretical interest attaching to the phenomenon of meiosis lies in the convincing proof which it affords of the existence of persistent *structural* units which are directly responsible for the appearance of the various characters manifested by the developing organism.

It was formerly held by many investigators that the significance of fertilisation consisted merely in doubling the amount of chromosome-substance, and that reduction merely paved the way for this addition of bulk. The mass was supposed to act as a whole, rather than to be made up of separate and independent units. All the results of recent investigations have

\* Much has been made of the fact that the chromosomes of successive cell generations can sometimes be almost certainly identified with those that went into rest during the previous telophase. But it should be remembered that such may easily happen, especially when divisions succeed each other rapidly, for the chromosome substratum chiefly consists of colloidal substance, which would only slowly lose its individuality under quiescent conditions.

† Farmer and Moore, 'Quart. Journ. Micr. Sci.,' vol. 48.

tended to upset this view, and to prove that just as the body itself is made up of individual cells, each an entity in itself, so also, and even more emphatically, the permanent elements of the nucleus are structurally distinct and physiologically diverse.

Reduction or meiosis is no mere halving of the bulk of the chromosome-substance; its significance is to be discerned rather in the sorting out and distribution between two daughter cells of the structural entities—the primordia of characters—which are handed on from one generation to another.

We may now follow, as briefly as possible, the way in which the chromosomes behave during the meiotic divisions, and contrast the process with that which obtains during an ordinary or somatic fission.

In the first place it is observed that as the nucleus advances towards division, and the chromosomes are able to be identified within it, their number is only half of that characteristic of the nuclei of the preceding cell generations that have arisen from the fertilised egg. A careful study of the facts has shown that this reduction is as yet only a false one, and that the real process is far more complicated. For each apparent chromosome is in reality made up of *two* chromosomes which have become temporarily united together. This union is, for a time, very intimate, and we have good grounds for saying that it is not mere chance which determines which two particular chromosomes shall unite to form a pair. For example, it may happen that two chromosomes in a premeiotic nucleus are different from the rest. Sometimes there are several dissimilar pairs. At meiosis, the pairing always takes place in such a way that like joins with like, to form the pseudo-chromosomes, as these bodies have been well called.

During the earlier stages, each of the paired chromosomes divides longitudinally, as in an ordinary somatic division, but the two halves do not yet become separated from one another. When the time comes for the splitting asunder of the two daughter-chromosomes to form the two nuclei, we find that the paired individuals now disunite and travel apart. When this has taken place, and the two daughter nuclei have been formed, the longitudinal fission, to which I have already referred, finally takes effect, and the two nuclei at once proceed to divide again. Each chromosome splits along the line of cleavage already marked out during the previous division. The process essentially resembles that of a somatic fission, but with this important difference, *viz.*, that the *reduced* number of chromosomes is retained. And this feature continues to mark all the nuclei of the post-meiotic cells, until the number is again doubled at the next act of fertilisation.

The net result of the meiotic phase is to effect the reduction in the number of chromosomes, and hence of the chromomeres, by the device of distributing half of the entire duplicated stock to one, and half to the other, nucleus. I would remark, in passing, that this fact points to the conclusion that the

real importance of the chromosomes lies more in their *structure* as bundles of chromomeres than in their material composition alone.

I have referred to the peculiar circumstance that *similar* chromosomes pair to form the pseudo-chromosomes. It is, of course, evident that one of each of them will pass into the daughter-cells at meiosis, and hence ultimately into the nuclei of the gametes, which are the direct descendants of these cells. But it is also evident that when the gametes unite at fertilisation, not only will the chromosomes be doubled in number, but that the peculiar ones when present at all will again occur in pairs. The special interest of this lies in the indication that the chromosomes which pair at meiosis are derived from the male and female parents respectively.\* This being so, meiosis is seen to be invested with profound significance in relation to the sorting out of the structural elements originally contributed by the parents, and the distribution of entire sets of them, but *in different combinations*, into the sexual cells. Thus the facts of meiosis are seen to fall completely into line with the conclusions drawn from experiments on breeding as far as the numerical distribution of characters is concerned.

That this pairing of the chromosomes is a real fact, and not a mere fanciful interpretation put on a complex series of phenomena, is abundantly proved by the work of the last few years. I will give two examples in illustration. It is well known that hybrids are frequently sterile, and this is often found to be associated with the failure on the part of the organism to accomplish the reduction of the chromosomes satisfactorily at the meiotic phase. This may happen if the chromosomes contributed by the two parents are unequal in number, and Rosenberg showed that it occurs in the hybrid between two of our common Sundews. *Drosera rotundifolia* has 10, and *D. longifolia* has 20 chromosomes in the sexual nuclei. When these two species are crossed, the somatic cells of the hybrid will, of course, exhibit 30 (10+20) chromosomes in their nuclei. Hence, at meiosis, it might be expected that there would be 15 pseudo-chromosomes produced. Instead of this, the number is seen to be 20. But, on examination, the 20 prove to be made up of 10 large and 10 small ones. What has happened is that the 20 contributed by the one parent, as well as the 10 furnished by the other, have retained their autonomy, but the 10 from *D. rotundifolia* have united with 10 of the 20 belonging to *D. longifolia*. The small ones represent the remaining 10, for which there were no mates. The statement that at meiosis the pseudo-chromosomes are

\* I have purposely avoided discussing in detail the peculiar heterochromosomes that have been observed by Wilson and others in insects, as we are not at present in possession of sufficient evidence to fully estimate their possible significance.

composed of individuals derived from the male and female parents respectively could hardly receive stronger support.

The second example I will take from a hybrid fern which is at present under investigation in my laboratory. The plant in question, *Polypodium Schneideri*, is the result of a cross between *P. aureum* and *P. vulgare* var. *elegantissimum*. The nuclei of the former contain 35 chromosomes after meiosis, whilst *P. elegantissimum* has about 97 or 98. The chromosomes of the hybrid, at meiosis, instead of exhibiting a mean between the two parents, *i.e.* 66, has a much larger number. There is a little variation in the actual numbers, but the average lies between 90 and 100. That is to say, the quantity characteristic of the *elegantissimum* parent is retained. Besides this, we find at meiosis a large number of small, unpaired, chromosomes; these obviously represent the surplus over and above those furnished by the *aureum* parent. At division they betray their real nature by passing in their entirety to one of the daughter-nuclei, when these are formed.

Facts such as those I have recounted seem to emphasise the permanent individuality of the chromosomes, and indeed they have often been appealed to in this sense. The case is further strengthened by the not unfrequent occurrence in cells, before they have undergone meiosis, of one or more pairs of chromosomes that are different from the others, a circumstance to which I have already alluded.

Moreover, the mode of sorting out, and the general behaviour of the chromosomes throughout the cell-generations of the organism, are at first sight in striking accord with the results of statistical investigations on heredity, and they are looked upon by many people as representing the primordia of the characters which we have already considered.

But I believe there is a fatal objection to this point of view, and one which conclusively proves that we cannot regard the *chromosome itself* as the structural unit responsible for the characters of the organism. The objection is based on their relatively small numbers, and it was urged, though in a somewhat different connection, by Weismann, many years ago. I will illustrate my meaning by one example, and I wish to express my thanks to my friend, Mr. A. D. Darbishire, for kindly collecting the facts of the case. In the two pure races of *Pisum sativum* and *P. arvense*, there are no less than 18 pairs of characters respectively which in the hybrids behave as allelomorphs. Since, however, there are only seven chromosomes in the nuclei of the gametes, it is evident that the 18 allelomorphs could not all occur independently of each other, but that, if the chromosome be adopted as the unit, the allelomorphs would appear in at most seven groups, and further, that the members of each group would always appear in association.

Now such a correlation does sometimes occur in respect of a few characters, but it becomes relatively insignificant when the total number of independent pairs of allelomorphs is borne in mind. And if this causes difficulty in dealing with relatively few characters, it obviously gathers in force when the vast numbers of features which distinguish an organism are remembered.

But if for the chromosomes we substitute the *chromomeres* as representing the discrete units which are responsible for the production of the hereditary characters, the last objection loses its force. The chromomeres, consisting of special chromatin-containing linin bodies, are present in great quantities; so numerous that, as far as I am aware, they have never been counted in a single chromosome. They fulfil the condition of being distinct entities; and also, at meiosis, the divergent chromosomes contain, or rather are made up of, chromosomes arranged in two opposing groups, and derived from different parents.

At the same time, I am aware that there are certain difficulties introduced by this suggested substitution of the chromomeres for the chromosomes, though I think these are more apparent than real.

The cardinal facts which we have to take into account are: first, the large number of the allelomorphs and, secondly, their mutual independence. These two circumstances, taken together, are irreconcilable with a view that would regard the allelomorphs as invariably assigned to particular chromosomes. But if we attach to the chromomeres, or to any other still smaller particles, the properties of separate character-producing substances, the widely entertained view as to the real structural persistence of the chromosomes themselves will require some modification. For in order to give that complete independence observed to exist between most of the allelomorphs, it is clear that any given chromosome must be correspondingly indifferent as to which chromomeres enter into its composition.\* This, however, amounts to a surrender of the doctrine that there exists any permanent structural arrangement in an individual chromosome lasting from one generation to another.

They might, perhaps, be compared with the hands that are successively dealt out from a pack of cards: each new hand, in respect of the number of cards, may resemble, but is not really identical with, those of the preceding deals. So, too, the chromosomes which reappear at each division would be similar to, but not *necessarily* the same as, those of the preceding division. The material particles of which they are built up are shuffled in the intervals

\* In the heterotype pseudochromosomes, each member must, however, be composed of homologous primordia contributed by the male and female parent respectively. It may well be that this is the significance of synapsis, which forms so characteristic a feature of the heterotype mitosis.

elapsing between one division and another. This might well happen without any change of form ; just as in some crystals, for example, certain atoms or groups of atoms may be replaced without altering the crystalline form. In the organic world, we know that the precise form of a particular organ may be produced, although the cells of which it is made up may not be those which should have entered into its composition. I need only refer to the experiments proving that a substitution of blastomeres may take place in the early stages of development of the frog's egg without affecting the subsequent differentiation of the tissues. Again, I might refer to the replacement of the crystalline lens, when extirpated from a salamander's eye, by a new lens formed not from ectodermal, but from mesodermal, cells. And, as a matter of fact, the abundant anastomoses which the linin exhibits during the earliest stages of nuclear division seem to provide just that mechanical condition for distribution which theory requires. This anastomosis has always been a difficulty in the face of assumptions of chromosomal permanence.

The chromosomes, then, would represent similarly *organised* groups of chromomeres, but they would *not* necessarily represent permanent or persistent structures in the sense that each one is to be looked on as being invariably composed of the same chromomeres. Their constancy in form and number would be the expression of *organisation* within the cell, and not of an unchanging aggregation of the *same constituents*.

The relation between the chromosomes and the origin of them from the male or female parent respectively of course remains unaffected ; what evidence we possess points to the conclusion that the two sets of chromosomes, and consequently their chromomeres, remain distinct in all the cell-generations up to meiosis.\* In a number of animals and plants they reappear in two distinct groups at every nuclear division during the earlier stages of ontogeny ; and Blackman has shown, for the Uredineæ, that the nuclear approximation which corresponds to fertilisation is not really consummated until many cell-generations afterwards ; not, indeed, until the meiotic phase comes on. In these plants, then, the chromosomal isolation is carried to an extreme, for the sexual nuclei do not unite during the somatic phase to form a single nucleus ; they co-exist side by side as separate bodies, although they periodically divide in a synchronous fashion.

In suggesting the chromomeres as the agents which are responsible for the production of the characters, I am aware that I may be laying myself open to the charge of merely retreating from a position never very strong, and now

\* Even if it should be found that this distinctness is definitely lost in the premeiotic nuclei, it would not weaken the strong evidence in favour of the shuffling of the primordia, and their re-arrangement in groups of homologous pairs, at meiosis.

beginning to prove untenable, to another that will perhaps turn out to be no less vulnerable. But I think we are bound to take into account the structures we can actually see, and the evolutions of which we can definitely follow, before we take refuge in other and merely hypothetical entities. At any rate, the plain facts of heredity, on the one hand, seem to postulate the existence of large numbers of real units in the cell in order to account for the observed phenomena, and, on the other hand, a study of the cell shows that such structures are really present, although they may be crowded together into larger or smaller distinct packets. And this will continue to be true, whether the chromomeres, or still smaller particles, are adopted as the units.

This view as to the variable nature of the chromosomes seems to me to render intelligible certain other facts which have often proved puzzling, and have become especially so during some recent investigations on the cytology of ferns. In studying the nuclei of a series of varieties that have sprung, and are still originating, from some of our common species, *e.g.*, the Male Fern, one cannot fail to be struck by the somewhat unexpected occurrence of chromosome-numbers which show a great, but quite irregular, deviation from that characteristic of the type.

Sometimes the new numbers are in excess, sometimes they fall below that which one would have anticipated. Thus in two varieties of the Male Fern known as *Polydactyla*, the numbers in the prothallial cells are 66 and 90 respectively. The two ferns are remarkably alike in external features, as is indicated by their common name; but they are known to have originated as distinct plants. In spite of their similarity, they show many points of important difference when the various stages of their life-histories are compared. They are both apogamous, and both replace the normal act of fertilisation by the union of nuclei derived from adjacent vegetative cells, in spite of the fact that they produce perfectly active sperms. One of them, however, entirely lacks the female organ—the archegonium—whilst in the other, though present, it is quite functionless. Along with these peculiar features there is associated the change in the number of the chromosomes already referred to. Now, it is important to notice that the differences do not form multiples either of each other or of the number (72) of the typical fern from which they are known to have originated. And when other varieties are brought into the comparison, all grades of deviation are shown. It would thus appear, since there is no corresponding diversity in the sizes of the individual chromosomes, as though the variation must have arisen through a *re-arrangement of the whole substance* of which they are made up; in other words, the chromomeres have become thrown into new combinations quite apart from any further modifications they may have undergone. The



consideration of such an example as this serves to illustrate the truth of the general statement that the chromosomes, like other organs, are subject to change, both as to size and number. Such a difference is not uncommon between closely allied species; and in several of the ferns to which I have referred, the number is not even constant in the same individual. It would seem, then, that the bodies in question form rather a frail support for the heavy weight of speculation that has been piled upon them.

Much of the importance which has been attached to the chromosomes in connection with organisation is connected with the fact that in the plants the process of meiosis is not directly followed, as it is in animals, by the differentiation of sexual cells. In most plants the meiotic phase forms the starting point of a definite stage in the life-history of the organism, and all the cells are distinguished by the possession of the half, or the post-meiotic, number of chromosomes. Thus in the fern, the plant as we know it, possesses the double set. Meiosis is associated with the formation of the spores, and when these germinate they give rise to the sexual plant, the prothallium. All the cell-nuclei of this structure continue to show the *reduced* or *post-meiotic* number of chromosomes as the consequence of meiosis. It seemed natural, then, to regard this change of nuclear structure as connected in some causal way with that alternation of a sexual and a sexless generation which is so striking a feature in the vegetable kingdom, especially in the higher classes, of which the ferns may be taken as examples.

The investigations on apogamy, and especially on apospory, have shown this view to be untenable. Indeed, it has been known for some years that eggs of species of Echinoderms when stimulated to develop parthenogenetically proceed to segment on perfectly normal lines in so far as they can develop at all. Their nuclei retain the post-meiotic number of chromosomes throughout the short life of the early larval stages, that is up to the death of the embryonic animals.

We have been able to show, in the case of ferns, that in apospory there may be a direct transition from the fern plant to the prothallium, accompanied by the *total suppression* of the meiotic phase which normally marks the passage from the one generation to the other. The nuclear character of such an aposporous prothallium exactly resembles that of the fern plant from which it sprang, instead of only having half the chromosomes. The chief difference of importance which the prothallium itself exhibits, as contrasted with an ordinary one, lies in its inability to produce fertilised eggs. The sexual organs may be fully formed, and the sperms are frequently found to be vigorously motile. Fertilisation, however, never occurs, and the fern either arises directly from the unfertilised egg, or from the tissues of the

prothallium. In such a case, then, the entire life-history is passed through without any reduction of the chromosomes; the egg, like the other cells of the prothallium, already contains the full complement, which normally is not supplied until fertilisation. There is, therefore, no reason to be surprised at the omission of fertilisation in these cases.

It is sometimes possible to induce prothallia which are normal as regards their nuclear contents, *i.e.*, are reduced, to produce embryos apogamously. But in all these instances, excepting one, we have found that the normal sexual fusion, which is here prevented from taking place, is replaced by a fusion of nuclei from adjacent vegetative cells. In this way the normal doubling of the chromosomes is secured. This circumstance has been used as an argument to prove that at any rate the double set is necessary for the production of the sporophyte generation.

We have encountered one instance, however, in which there seems no room for doubt that the fern plant sprang from a prothallium of *post-meiotic* structure, without any preparatory nuclear fusion. The plant in question\* appeared in a close fernery, and, from what we know of its history, it must have started as a spore. Presumably, therefore, it had passed through meiosis, and this is borne out by the small number of chromosomes present in its nuclei. From the prothallium, fern plants were produced apogamously, and they are all characterised by the possession of the *reduced*, *i.e.*, the post-meiotic, number of chromosomes.

It thus becomes evident that, given a *complete set of normal* chromosomes, whether in single or in duplicate, the entire life-history *may* be passed through. The peculiar fusions between the nuclei of adjacent prothallial cells, together with the extreme rarity of the formation of the fern plant without the double set, may perhaps be taken to indicate some *normal* relation as existing between the duplicate number and the sporophyte, but it clearly cannot be of any fundamental importance.

The fact that both stages of the life-history can be gone through in the absence of that nuclear change which ordinarily corresponds to each one of them is a proof that the organisation of the species, whatever may be the phases through which it passes, can be completely effected *either* with the single, *or* with the double, set of chromosomes; but the meaning attached to sexuality, as importing into the organism the means of producing variation, becomes even more clearly defined than before.

I desire, in conclusion, to obviate a possible misapprehension which, perhaps, might arise from the way in which I have spoken of the chromomeres as the agents responsible for the appearance of the characters

\* *Lastrea pseudo-mas cristata apospora*, see 'Annals of Botany,' vol. 21, p. 180.

manifested during ontogeny. Of course, in calling them *primordia*, I only mean to imply that they are the agents that determine the particular sequence of chemical changes which shall occur in the unstable cytoplasm. I do *not* mean that they are themselves the characters *in parvo*. The very fact that, as agents, they may persist in a dormant state, and thus fail to excite the appearance of the characters appropriate to them, as may happen in the concurrent presence of dominant and recessive allelomorphs, sufficiently emphasises this. For as long as the course of metabolism can be completely impelled by one of the two allelomorphs, the influence of the other will remain latent. We may, perhaps, find an analogy in the behaviour of *Penicillium*, which, although it is provided with a powerful battery of ferments, does not indiscriminately attack all the nutrient materials of the substratum when plentifully provided with suitable carbohydrates.

Such an example serves also to illustrate another aspect of the problem, and one that perhaps often meets with less attention than it deserves. For admitting that there are good grounds for assigning the production of hereditary characters to the action of discrete bodies, we are still without an explanation of the obvious facts of functional correlation and adaptation. The environment, in the widest sense of the term, often determines *which* of the many potential characters of a cell shall actually develop.

We know something as to the manner in which this may be achieved in certain instances. For example, nutritional, as well as other and more obscure, stimuli were found by Klebs to determine the particular one out of several alternative courses of development which was followed in the case of certain algæ. And again, the Hormones of Starling may be quoted as types of substances, apparently of relatively simple chemical structure, which serve as excitors of specific cellular activities in the complex animal body.

The conceptions of *primordia* which are responsible for the appearance of hereditary characters on the one hand, and also of *specific exciting substances* on the other, seem to be both necessary, and the one to supplement the other.

Every organism and every cell has larger potentialities than are ever realised in any single ontogeny. The hereditary mechanism imposes the limits within which development *can* take place; but within those limits other conditions may determine the path actually followed.

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